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TITLE: Prevalence and Characterization of BRCA2 in Male Breast Cancer Cases

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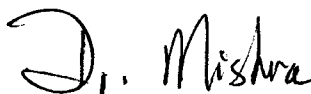
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13. ABSTRACT (Maximum 200 words) <p>Male breast cancer (MBC) is rare, with an incidence rate of 0.5-1/100,000 per year. The objective of this grant is to study a series of unselected population-based MBC cases to characterize the role of <i>BRCA2</i> in MBC and to estimate the attributable risk of MBC due to <i>BRCA2</i> mutations. At the end of this third year of funding, we have collected DNA samples on 141 MBC cases and paraffin-embedded tissue on 21 of those. Of the 97 MBC cases with available family history data, 52 (54%) have a family history of breast cancer in first or second degree relatives. To detect germline mutations in <i>BRCA2</i>, single strand conformational analysis (SSCA) of the DNA samples is being performed for seventy-four amplicons spanning the entire coding region and intron/exon boundaries. All but one amplicon has been screened for mutations for the samples. We have identified frameshift, missense, silent, and non-coding mutations, and polymorphisms. We are currently examining whether the missense and non-coding mutations are likely polymorphisms. The actual mutations and estimated prevalence are unpublished, as yet, and are presented in the body of the progress report.</p>				
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Susan L. Neuhansen
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Annual Progress Report Grant DAMD-17-96-I-6266

Period: June 15, 1998-June 15, 1999

INTRODUCTION

Breast cancer is a rare disease in men, affecting approximately 0.1% over their lifetime, as compared to 12% in women. However, despite the difference in prevalence, male and female breast cancers are similar in presentation and response to treatment. In families with multiple cases of female breast cancer and a male breast cancer, we observed that these families were not linked to *BRCA1* (Stratton et al., 1994), the first gene found to predispose to breast cancer. Using these same families, we localized *BRCA2* (Wooster et al., 1994), a second gene predisposing to breast cancer. *BRCA2* was cloned in 1995 (Wooster et al., 1995; Tavtigian et al., 1996). We were interested in the proportion of male breast cancer (MBC) attributable to mutations in *BRCA2* and estimated that 10-15% of male breast cancer could be caused by *BRCA2* mutations. In a study of loss of heterozygosity (LOH) of markers spanning the *BRCA2* gene, 16 of 24 (67%) MBC cases showed LOH in at least one of the two markers, suggesting a role for *BRCA2* in the development and/or progression of MBC (Prechtel et al., 1998). Screening for mutations in *BRCA2* in male breast cancer cases has been performed by several groups. Seven (21%) *BRCA2* germline mutations were reported in a Swedish study of 34 MBC patients. Of those seven cases, only one had a family history of breast cancer (Haraldsson et al., 1998). In a British study of 28 MBC cases, 2 (7%) deleterious mutations were identified (Mavraki et al., 1997). In a US study of 54 MBC cases, two (4%) cases had *BRCA2* mutations, and one of them had a family history of breast cancer (Friedman et al., 1997). The remaining eight cases with a family history of breast and/or ovarian cancer did not have a mutation in either *BRCA1* or *BRCA2*. In a study of 18 Hungarian male breast cancer cases, 6 of 18 (33%) had truncating mutations in *BRCA2* and no mutations in *BRCA1* (Csokay et al., 1999). Combining all four of these studies, the proportion of MBC cases, unselected for a family history of breast cancer, with a germline mutation in *BRCA2* is 12.7%.

The objective of this grant (proposed prior to cloning of *BRCA2*) is to study MBC cases to characterize the role of *BRCA2* in MBC and to estimate the attributable risk of male breast cancer due to *BRCA2* germline mutations. This is a population-based study. The results to date are discussed below, as well as future work to be performed.

BODY

Our primary goals for the past year included continued ascertainment of additional male breast cancer cases and screening for mutations in the *BRCA2* gene.

Progress in ascertainment of male breast cancer cases (Technical objective 1 - tasks 1-4):

During the past year, we continued to enroll MBC cases in Utah through the Utah Cancer Registry (UCR) and through the Wyoming Cancer Registry. Rapid-reporting of recently diagnosed cases resulted in ascertainment of 5 Utah cases in the last year. Two MBC cases from

Wyoming are participating, one has been sent a blood kit and questionnaire, and we are still trying to contact 8 men. The Colorado Cancer Registry and Idaho Cancer Registry have agreed to participate, but they have sent no request letters. We received IRB permission and posted our study on the website of a Male Breast Cancer Support Group, inviting all males who have had breast cancer to participate in our study. Four MBC cases have been recruited from the web site.

We currently have DNA samples from 141 MBC cases (Table 1). The age at diagnosis ranges from 28-93 years. Of the 94 MBC cases with family history data, 52% have a family history of breast cancer in at least one first degree relative. Questionnaires have been sent to 65 of the MBC cases we collected from Utah, Wyoming, or the WWW and 33 have been returned. Unfortunately, nine men have died since we obtained their blood samples.

Table 1. Source of male breast cancer cases.

Source	# Cases	+family history	- family history	Unknown family history
Univ of Utah	59	22	29	8
Wyoming	2	0	2	0
WWW site	4	2	2	0
Chicago	5	2	2	1
ICRF	40	4	0	36
MSKCC	27	17	10	0
Texas	2	0	0	2
Italy	2	2	0	0
Total	141	49	45	47

Status of Technical objective 2 (Tasks 5-7): Characterization of loss of heterozygosity (LOH). The original intent of this objective, designed prior to cloning *BRCA2*, was to classify the MBC cases as likely carrying a *BRCA1* or *BRCA2* mutation based on loss of chromosomal segments in the regions containing *BRCA1* and *BRCA2*. However, *BRCA2* was cloned prior to funding of the grant, so that we proceeded directly to screening for mutations in the MBC cases. We are still interested in this objective to correlate the LOH results with mutation screening results. However, we are waiting to acquire additional tissue blocks.

Status of Technical objective 3 (Tasks 8-10): Fine-structure haplotype construction and analysis. We have decided not to perform the tasks associated with this objective as it is not necessary because we can easily screen for mutations in *BRCA2*. When the grant was submitted, *BRCA2* had not yet been cloned, so it would have allowed us to begin characterizing individuals

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as to the likelihood of carrying a mutation. However, when the grant was funded, *BRCA2* had been isolated so that we could begin mutation screening, which is more productive and efficient than performing the tasks related to Technical Objective 3.

Progress in screening for *BRCA2* mutations (Technical objective 4 - tasks 11-12). During the past year, we have screened the MBC set for mutations in *BRCA2*. Single strand conformational analysis (SSCA) was performed on 73 amplicons, with an average size of 250 base pairs and a maximum size of 300 base pairs. We are still working on amplifying the 3' end of exon 27 - several primer pairs have not worked. In Table 2, the results of the mutation screening are shown. One individual had three variants, two silent and 1 missense (all likely polymorphisms). These three variant may be in linkage disequilibrium. Ten frameshift mutations have been identified in 15 MBC cases, including 5 cases with the 6174delT founder mutation (Neuhausen et al., 1996).

Four missense mutations of unknown functional significance and three which are likely polymorphisms were identified. Observations which suggest that a missense mutation is not causal are: 1) the mutation is observed in a control group in equal or greater prevalence to an at-risk group; 2) there is not cosegregation of the variant and disease in a family with significant disease; 3) the missense change results in replacement of a similar amino acid to the wild-type protein; 4) there is not conservation of the wild-type amino acid between the human, mouse, and dog *BRCA1* or *BRCA2* proteins; and 5) the mutation does not occur in a putative functional domain. We will try to classify the four missense mutations as likely polymorphisms or undetermined based on the criteria listed. We will examine the frequency of each missense mutation in a set of 200 (400 chromosomes) unrelated DNA samples to see if this appears to be a polymorphism based on frequency in an unaffected population (frequency > 1%).

For the splice and two non-coding mutations, we will try to obtain another blood sample in order to extract RNA and determine if either an exon is skipped or intronic sequence is transcribed. The K3326 mutation leads to truncation of the protein, yet it is a polymorphism - it would appear that mutations in the last exon of the coding region of the gene are not deleterious. Seven other polymorphisms were identified and will be useful in other studies to determine the age of founder mutations.

Therefore, of the mutations known to be deleterious, i.e. those that result in a truncated protein product, there were 15/141 or 10.6%. If one considers that the sensitivity of SSCA is approximately 80%, then the prevalence would be 13.2%. This is still a conservative estimate, because we do not have data on all samples for all amplicons. A number of samples dropped out for a varied number of amplicons. They will be repeated and then we will recalculate the prevalence. Currently, there are 9 samples which dropped out on more than 50% of the amplicons and they include 2 samples from Italy, one sample from Texas, 5 samples from MSKCC, and 2 samples from Utah. We will likely not be able to obtain more DNA from the non-Utah samples, so that the total number screened for the calculation of prevalence may be less than 141. Five mutation carriers have a positive family history, two have a negative family history, and 7 have an unknown family history. This suggests that family history is not a good predictor of the likelihood of carrying a *BRCA2* mutation.

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Table 2. *BRCA2* mutations identified in MBC cases

Mutation	Description of nucleotide change	Type of mutation	# observations or % for polymorphisms
1002delAA	del AA	Frameshift	1
2158delA	del A	Frameshift	1
4075delGT	del GT	Frameshift	1
4359ins6	ins TGAGGA	Frameshift	1
6174delT	del T	Frameshift	5
6175delG	del G	Frameshift	1
8804delA	del A	Frameshift	1
8822insT	ins T	Frameshift	1
9325insA	ins A	Frameshift	2
9481insA	ins A	Frameshift	1
G49L	G>T	Missense	1
S2247G	A>G	Missense	1
T1505A	A>G	Missense	1
T1915M	C>T	Missense	2
A2466V	C>T	Missense - likely polymorphism	1*
D1420Y	G>T	Missense - likely polymorphism	3
N991D	A>G	Missense-likely polymorphism	4
IVS16-14T>C	T>C	Non-coding	1
IVS8+56C>T	C>T	Non-coding	1
IVS2+1G>A	G>A	Non-coding Splice	1
203G>A	G>A	Polymorphism	18%
3' UTR	A>C	Polymorphism	22%
3' UTR	delT	Polymorphism	15%
K1132K	A>G	Polymorphism	23%
IVS21-66T>C	C>T	Polymorphism-non-coding	52%
K3326X	A>T	Polymorphism- STOP	1
3' UTR	A>G	Polymorphism-likely	3
L1522L	G>A	Silent	1*
S646S	C>T	Silent	1
V2171V	C>G	Silent	1*

* Same individual had three variants.

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Male breast cancer has been reported in *BRCA1*, albeit rarely. Because the majority of our MBC cases did not have mutations in *BRCA2*, we decided to screen for mutations in *BRCA1* in DNA from 56 Utah samples for which no deleterious *BRCA2* mutations were found. The SSCA screening is complete and the gels are currently being examined to identify variant bands. During the next year, we will sequence the variant bands to identify any *BRCA1* mutations.

Plans for the final year of funding. During the next year, our focus will be 1) to obtain questionnaires from the remaining participants; 2) determine whether the missense mutations are likely polymorphisms and whether the splice and non-coding mutations are functional; 3) sequence SSCA variants from the *BRCA1* mutation screening to identify mutations; 4) rescreen samples on specific amplicons which dropped out to complete the mutation screening; 5) correlate the findings for LOH with BRCA mutation results; and 6) try to extend those families where frameshift mutations were identified. We will also try to obtain an additional 30 MBC cases from outside Utah. We are working to establish a collaborative case-control study to examine variants in putative low penetrance genes which may affect the risk of breast cancer, including those genes involved in hormone metabolism, such as the estrogen and progesterone receptors. These genes would confer a low risk for breast cancer, yet because they are common, they would confer a higher attributable risk than the rare *BRCA1* and *BRCA2* variants. Researchers from institutions worldwide have agreed to participate, but we have only received 12 samples for that study, in addition to our current samples.

KEY RESEARCH ACCOMPLISHMENTS:

- o Collecting the largest single-site set of MBC cases
- o Identification of *BRCA2* mutations in MBC cases

REPORTABLE OUTCOMES:

Presentation: "Genetic Epidemiology of Male Breast Cancer" at the Breast Cancer Linkage Consortium Meeting, October, 1998.

CONCLUSIONS:

Male breast cancer is a relatively rare disease, as shown by our difficulty in rapidly accruing a large number of living cases for this study. *BRCA2* mutation screening was completed on 141 MBC cases. Fifteen known deleterious mutations were identified (frameshift mutations which caused premature protein termination) for a prevalence of 11%. Because the sensitivity of SSCA is likely 80%, the population prevalence would be 13.2%. It does not appear that family history is a good predictor of *BRCA2* mutation status. *BRCA2* mutations appear to be more prevalent in unselected MBC cases than in unselected female breast cancer cases.

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